IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Marc Vidal et al. Art Unit : Unknown
Serial No. : Examiner : Unknown
Filed : HEREWITH
Title : REVERSE TWO-HYBRID SYSTEMS

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the specification:

Page 1, between line 3 and line 4, please insert:

-- RELATED APPLICATIONS

This application is a continuation of (and claims the benefit of priority under 35 USC 120) of U.S. Application Serial No. 09/620,680, filed on July 20, 2000, which is a continuation of U.S. Application No.09/300,839, filed April 28, 1999, now abandoned, which is a continuation of U.S. Application Serial No. 08/923,274, filed September 4, 1997, now U.S. Patent No. 5,955,280, issued September 21, 1999, which is a continuation of U.S. Application Serial No. 08/959,536, filed October 24, 1997, now U.S. Patent No. 5,965,386, issued October 12, 1999, which is a continuation of U.S. Application Serial No. 08/420,525, filed April 11, 1995, now abandoned, all of which are incorporated herein by reference.--

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Replace the paragraph at page 33, line 28 with the following:

--Fig. 2 is a map of the plasmid p2.5. A portion of pPC97 (left panel) containing a polylinker, is represented by SEQ ID NO: 7. The amino acid sequence encoded by this portion of pPC97 is represented by SEQ ID NO: 8. A portion of pPC86 (right panel), containing a polylinker, is represented by SEQ ID NO: 9. The amino acid sequence encoded by this portion of pPC86 is represented by SEQ ID NO: 10.--

Replace the paragraph at page 35, line 2, with the following:

--Fig. 10A is a schematic representation of plasmids into which the CYH2 counterselectablemarker was inserted. A portion of pPC97 (left panel), containing a polylinker, is represented by SEQ ID NO: 7. The amino acid sequence encoded by this portion of pPC97 is represented by SEQ ID NO: 8. A portion of pPC86 (right panel), containing a polylinker, is represented by SEQ ID NO: 9. The amino acid sequence encoded by this portion of pPC86 is represented by SEQ ID NO: 10.--

Replace the paragraph at page 37, line 31 with the following:

--Fig. 21 is a schematic representation of the Marked Box 2 domain and the mutations obtained with the two-step selection method. The amino acid sequences of the Marked Box 2 domains of E2F5, E2F4, E2F2, and E2F1 are represented by SEQ ID NOS: 11-15, respectively. The amino acid sequences of the Marked Box 2 domains of the alleles E2F1-20, E2F1-30, E2F1-32, and E2F1-65 are represented by SEQ ID NOS: 16-19, respectively.--

Replace the paragraph at page 44, line with the following:

--Construction of Plasmids for Producing Hybrid Proteins: Plasmids p97.CYH2 and pMV257 are useful in the invention for producing hybrid proteins having a GAL4-DB or AD, respectively, fused to a potential interacting molecule of interest (Fig. 10B). These plasmids are produced by inserting a sequence encoding CYH2 into pPC97 (for DB plasmids) or pPC86 (for AD plasmids) (Fig. 10A). Both p97.CYH2 and pMV257 have (*i*) a yeast *ARS4* origin of replication; (*ii*) a yeast *CEN6* centromeric sequence; (*iii*) a selectable marker (e.g., *LEU2* for pPC97, and *TRP1* for pPC86); (*iv*) a yeast *ADH1* promoter and terminator; (*v*) a GAL4-DB (for pPC97) or a GAL4-AD

(for pPC86); (*vi*) an SV40 large T antigen sequence encoding a nucleolar signal sequence positioned in frame with the DB or AD domain; (*vii*) a bacterial origin of replication; and (*viii*) a *CYH2* counterselectable marker. Those skilled in the art recognize that numerous similar plasmids can be used to produce hybrid proteins. For example, hybrid proteins that include the DB or AD of VP16 (from Herpes Simplex Virus or Ace1 can be produced with plasmids having, in place of the GAL4-DB or -AD, sequences encoding the VP16 or Ace1 DB or Ace1 AD. Similarly selectable markers other than *Leu2* and *Trp1* can be used. These plasmids can be constructed with conventional molecular biology methods. Generally, in order to select for a yeast cell containing one of these plasmids, the yeast cell should not, in the absence of the plasmid, express a functional gene product which corresponds to the selectable marker. For example, a yeast cell into which p97.CYH2 is transformed should have a *leu2* mutation; thus, a transformant containing p97.CYH2 can be selected on a medium which lacks leucine. The yeast strains MaV103 and MaV203 are particularly useful in conjunction with p97.CYH2 and pMV257.--

At pages 81-82, delete the current Sequence Listing and substitute therefor the accompanying Sequence Listing as pages 81 through 87.

In the Claims:

Cancel claims 2-107 without prejudice.

Add the following new claims 108-148.

--108. A method for determining whether a first test protein interacts with a second test protein, said method comprising:

a) providing in a cell:

i) a counterselectable reporter gene operably linked to a first DNA binding protein recognition site or a selectable/ counterselectable reporter gene operably linked to a first DNA binding protein recognition site;

ii) a first fusion gene which expresses a first hybrid protein, said first hybrid protein comprising a test protein covalently bonded to a DNA binding moiety which specifically binds to said DNA binding protein recognition site;

iii) a second fusion gene which expresses a second hybrid protein, said second hybrid protein comprising a test protein covalently bonded to a gene activating moiety; and

b) detecting expression of said reporter gene as a measure of the ability of said first test protein to interact with said second test protein.

109. The method of claim 108, wherein said cell further comprises a second reporter gene.

110. The method of claim 109, wherein said cell further comprises a third reporter gene.

111. The method of claim 109, wherein said second reporter gene is a counterselectable reporter gene.

112. The method of claim 109, wherein said reporter genes are different.

113. The method of claim 109, wherein said reporter genes are identical.

114. The method of claim 109, wherein said second reporter gene is operably linked to a second DNA binding protein recognition site.

115. The method of claim 114, wherein said first and said second DNA binding protein recognition sites are identical.

116. The method of claim 114, wherein said first and said second DNA binding protein recognition sites are different.

117. The method of claim 115, wherein said reporter genes are different.

118. The method of claim 117, wherein said reporter genes are operably linked to different promoters.

119. The method of claim 118, wherein a fusion gene is located on a plasmid.

120. The method of claim 119, wherein said plasmid is a low copy number plasmid.

121. The method of claim 118, wherein said reporter gene is integrated into the genome of said cell or is located on a plasmid.

122. The method of claim 118, wherein said cell is a yeast cell.

123. The method of claim 122, wherein said yeast cell is *S. cerevisiae*.

124. The method of claim 108, wherein said counterselectable reporter gene is selected from the group consisting of URA3, LYS2, CYH2, CAN1, and GAL1.

125. The method of claim 108, wherein the number of said DNA binding recognition sites is between 1 and 100.

126. The method of claim 125, wherein the number of said DNA binding recognition sites is between 1 and 20.

127. The method of claim 108, wherein said counterselectable reporter gene is detected as inhibition of growth.

128. The method of claim 108, further comprising isolating a cell which expresses said reporter gene.

129. The method of claim 128, wherein a fusion gene of said isolated cell is amplified.

130. The method of claim 128, wherein a fusion gene of said isolated cell is sequenced.

131. A cell comprising:

a) a counterselectable reporter gene operably linked to a first DNA binding protein recognition site;

b) a first fusion gene which expresses a first hybrid protein, said first hybrid protein comprising a test protein covalently bonded to a DNA binding moiety which specifically binds to said DNA binding protein recognition site; and

c) a second fusion gene which expresses a second hybrid protein, said second hybrid protein comprising a test protein covalently bonded to a gene activating moiety.

132. The cell of claim 131, wherein said cell further comprises a second reporter gene.

133. The cell of claim 132, wherein said second reporter gene is a counterselectable reporter gene.

134. The cell of claim 132, wherein said second reporter gene is operably linked to a second DNA binding protein recognition site.

135. The cell of claim 134, wherein said first and said second DNA binding protein recognition sites are identical.

136. The cell of claim 135, wherein said reporter genes are different.

137. The cell of claim 136, wherein said reporter genes are operably linked to different promoters.

138. A cell comprising:

i) a first reporter gene operably linked to a first DNA binding protein recognition site; and

ii) a second reporter gene operably linked to a second DNA binding protein recognition site;

wherein said first and said second DNA binding protein recognition sites are different.

139. The cell of claim 138, wherein a reporter gene is a counterselectable reporter gene.

140. A genetic construct comprising a fusion gene which expresses a hybrid protein, said hybrid protein comprising a test protein covalently bonded to a DNA binding moiety and a C-terminal tag.

141. A method for decreasing the occurrence of false positive interactions between a first test protein and a second test protein, said method comprising

a) providing in a cell:

i) at least two different reporter genes each operably linked to a different promoters having identical binding protein recognition sites;

ii) a first fusion gene which expresses a first hybrid protein, said first hybrid protein comprising a test protein covalently bonded to a DNA binding moiety which specifically binds to said DNA binding protein recognition site;

iii) a second fusion gene which expresses a second hybrid protein, said second hybrid protein comprising a test protein covalently bonded to a gene activating moiety;

b) maintaining the level of expression of said first and said second hybrid proteins at physiologically relevant levels;

c) detecting expression of a reporter gene as a measure of ability of said first test protein to interact with said second test protein.

142. A method for determining whether a test compound affects binding between a first test protein and a second test protein, said method comprising:

a) providing in a cell:

i) a counterselectable reporter gene operably linked to a first DNA binding protein recognition site or a selectable/ counterselectable reporter gene operably linked to a first DNA binding protein recognition site;

ii) a first fusion gene which expresses a first hybrid protein, said first hybrid protein comprising a test protein covalently bonded to a DNA bonding moiety which specifically binds to said DNA binding protein recognition site;

iii) a second fusion gene which expresses a second hybrid protein, said second hybrid protein comprising a test protein covalently bonded to a gene activating moiety;

b) contacting said cell with a test compound; and

c) detecting expression of said reporter gene as a measure of the ability of said compound to effect binding between said first and said second test proteins.

143. The method of claim 142, wherein said counterselectable reporter gene is selected from the group consisting of URA3, LYS2, CYH2, CAN1, and GAL1.

144. The method of claim 142, wherein said first fusion gene or said second fusion gene is derived from a cDNA library.

145. A method for determining whether a first test RNA molecule interacts with a test protein, said method comprising:

a) providing in a cell:

i) a counterselectable reporter gene operably linked to a first DNA binding protein recognition site or a selectable/ counterselectable reporter gene operably linked to a first DNA binding protein recognition site;

ii) a first fusion gene which expresses a first hybrid RNA molecule, said RNA molecule comprising said test RNA molecule covalently bonded to a first non-random RNA molecule;

iii) a second fusion gene which expresses a first hybrid protein, said first hybrid protein comprising a DNA binding moiety which specifically binds to said DNA binding protein recognition site, said DNA binding moiety being covalently bonded to an RNA binding moiety, wherein said RNA binding moiety specifically binds to said non-random RNA molecule;

iv) a third fusion gene which expresses said test protein covalently bonded to a gene activating moiety;

b) detecting expression of said reporter gene as a measure of the ability of said test RNA molecule to interact with said test protein.

146. The method of claim 145, wherein said ability of said first test RNA molecule and said test protein to interact is measured in the presence of a test compound.

147. A method for determining whether a first test RNA molecule interacts with a second test RNA molecule, said method comprising:

a) providing in a cell:

i) a counterselectable reporter gene operably linked to a first DNA binding protein recognition site or a selectable/ counterselectable reporter gene operably linked to a first DNA binding protein recognition site;

ii) a first fusion gene which expresses a first hybrid RNA molecule, wherein said first hybrid RNA molecule comprises said first test RNA molecule covalently bonded to a first non-random RNA molecule;

iii) a second fusion gene which expresses a first hybrid protein, said first hybrid protein comprising a DNA binding moiety which specifically binds to said DNA binding protein recognition site, said DNA binding moiety being covalently bonded to a first RNA binding moiety which specifically binds to said first non-random RNA molecule;

iv) a third fusion gene which expresses a second hybrid RNA molecule wherein said second hybrid RNA molecule comprises said second test RNA molecule covalently bonded to a second non-random RNA molecule;

v) a fourth fusion gene which expresses a gene activating moiety covalently bonded to a second RNA binding moiety which specifically binds to said second non-random RNA molecule;

b) detecting expression of said reporter gene as a measure of the ability of said first test RNA molecule to interact with said second test RNA molecule.

148. The method of claim 147, wherein said ability of said first and said second RNA molecule to interact is measured in the presence of a test compound.--

REMARKS

This Preliminary Amendment is submitted to place the application in better condition for examination and to facilitate the prosecution thereof. General support for new claims 108-148 can be found throughout the specification. Support for claim 108 can be found at page 5, line 12, through page 6, line 14. Support for claim 108 can be found at page 22, lines 7-10. Support for claim 110 can be found at page 32, lines 21-33. Support for claims 111-113 can be found at page 24, lines 2-5. Support for claims 114-115 can be found at page 23, lines 22-29. Support for claim 116 can be found at page 22, lines 19-22. Support for claim 117 can be found at page 24, lines 2-5. Support for claim 118 can be found at page 21, line 24 through page 22, line 13. Support for claim 119 can be found at page 19, lines 16-23. Support for claim 120 can be found at page 31, lines 14-16. Support for claim 121 can be found at page 19, lines 29-32. Support for claims 122-123 can be found at page 20, lines 13-16. Support for claim 124 can be found at page 26, line 11. Support for claims 125-126 can be found at page 23, lines 1-6. Support for claim 127 can be found at page 22, lines 4-6. Support for claims 128-130 can be found at page 76, line 25 through page 78, line 15. Support for claim 131 can be found at page 5, line 12 through page 6, line 15. Support for claims 132-134 can be found at page 24, lines 2-5. Support for claims 135-138 can be found at page 22, lines 19-22. Support for claim 139 can be found at page 24, lines 2-5 of the specification. Support for claim 140 can be found at page 14, lines 23-28. Support for claim 141 can be found at page 58, lines 31-33. Support for claim 142 can be found page 6, line 29 through page 7, line 20. Support for claim 143 can be found at page 26, line 11. Support for claims 144-145 can be found at page 9, line 23 through page 10, line 31. Support for claim 146 can be found at page 11, lines 2-8. Support for claim 147 can be found at page 11, line 11 through page 12, line 24. Support for claim 148 can be found at page 12, lines 26-30. No new matter has been added.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be examined. Please apply any charges or credits to Deposit Account No. 06-1050, referencing attorney docket no. 10974-239005.

Respectfully submitted,

Date: Dec. 21, 2001

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Version with markings to show changes made

In the specification:

Add the following at page 1, between line 3 and line 4,:

-- RELATED APPLICATIONS

This application is a continuation of U.S. Serial No. 08/923,274, filed September 4, 1997, now allowed; which is a continuation of U.S. Serial No. 08/420,525, filed April 11, 1995, now abandoned.

The paragraph beginning at page 33, line 28 has been amended as follows:

Fig. 2 is a map of the plasmid p2.5. A portion of pPC97 (left panel) containing a polylinker, is represented by SEQ ID NO: 7. The amino acid sequence encoded by this portion of pPC97 is represented by SEQ ID NO: 8. A portion of pPC86 (right panel), containing a polylinker, is represented by SEQ ID NO: 9. The amino acid sequence encoded by this portion of pPC86 is represented by SEQ ID NO: 10.

The paragraph beginning at page 35, line 27 has been amended as follows:

Fig. 10A is a schematic representation of plasmids into which the CYH2 counterselectablemarker was inserted. A portion of pPC97 (left panel), containing a polylinker, is represented by SEQ ID NO: 7. The amino acid sequence encoded by this portion of pPC97 is represented by SEQ ID NO: 8. A portion of pPC86 (right panel), containing a polylinker, is represented by SEQ ID NO: 9. The amino acid sequence encoded by this portion of pPC86 is represented by SEQ ID NO: 10.

The paragraph beginning at page 37, line 31 has been amended as follows:

Fig. 21 is a schematic representation of the Marked Box 2 domain and the mutations obtained with the two-step selection method. The amino acid sequences of the Marked Box 2 domains of E2F5, E2F4, E2F2, and E2F1 are represented by SEQ ID NOS: 11-15, respectively.

The amino acid sequences of the Marked Box 2 domains of the alleles E2F1-20, E2F1-30, E2F1-32, and E2F1-65 are represented by SEQ ID NOS: 16-19, respectively.

The paragraph beginning at page 44, line 8 has been amended as follows:

Construction of Plasmids for Producing Hybrid Proteins: Plasmids p97.CYH2 and pMV257 are useful in the invention for producing hybrid proteins having a GAL4-DB or AD, respectively, fused to a potential interacting molecule of interest (Fig. 10B). These plasmids are produced by inserting a sequence encoding CYH2 into pPC97 (for DB plasmids) or [pPC97] pPC86 (for AD plasmids) (Fig. 10A). Both p97.CYH2 and pMV257 have (*i*) a yeast *ARS4* origin of replication; (*ii*) a yeast *CEN6* centromeric sequence; (*iii*) a selectable marker (e.g., *LEU2* for pPC97, and *TRP1* for pPC86); (*iv*) a yeast *ADH1* promoter and terminator; (*v*) a GAL4-DB (for pPC97) or a GAL4-AD (for pPC86); (*vi*) an SV40 large T antigen sequence encoding a nucleolar signal sequence positioned in frame with the DB or AD domain; (*vii*) a bacterial origin of replication; and (*viii*) a *CYH2* counterselectable marker. Those skilled in the art recognize that numerous similar plasmids can be used to produce hybrid proteins. For example, hybrid proteins that include the DB or AD of VP16 (from Herpes Simplex Virus or Ace1 can be produced with plasmids having, in place of the GAL4-DB or -AD, sequences encoding the VP16 or Ace1 DB or Ace1 AD. Similarly selectable markers other than *Leu2* and *Trp1* can be used. These plasmids can be constructed with conventional molecular biology methods. Generally, in order to select for a yeast cell containing one of these plasmids, the yeast cell should not, in the absence of the plasmid, express a functional gene product which corresponds to the selectable marker. For example, a yeast cell into which p97.CYH2 is transformed should have a *leu2* mutation; thus, a transformant containing p97.CYH2 can be selected on a medium which lacks leucine. The yeast strains MaV103 and MaV203 are particularly useful in conjunction with p97.CYH2 and pMV257.

In the claims:

Claims 2-107 have been cancelled.